Department of Biochemistry University of Karachi

Intermediary Metabolism-II

Course NO: BCH-412
Credit hours (2+1)

Khwaja Ali Hasan

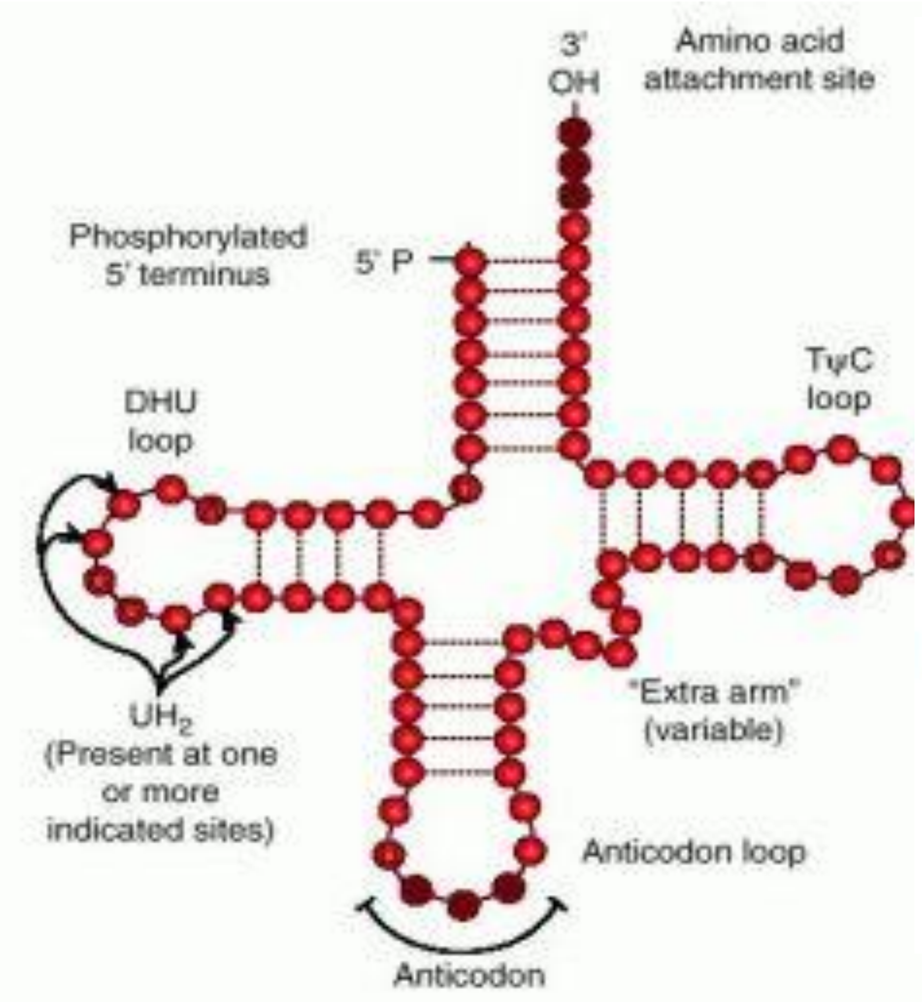
References and Books

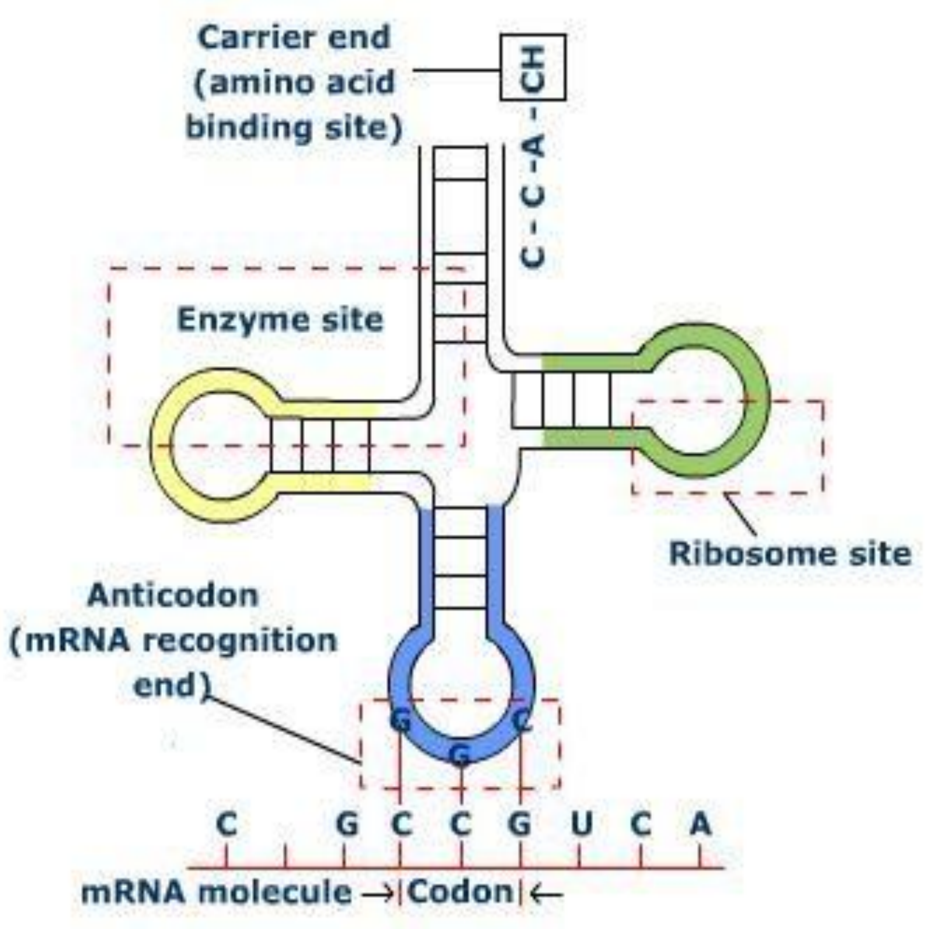
(1) Biochemistry by Jeremy M, Berg, Lubert Stryer 6th Edition

(2) Principles of Biochemistry (Lehinger) by David L. Nelson and Michael M. Cox, 5-7th Edition

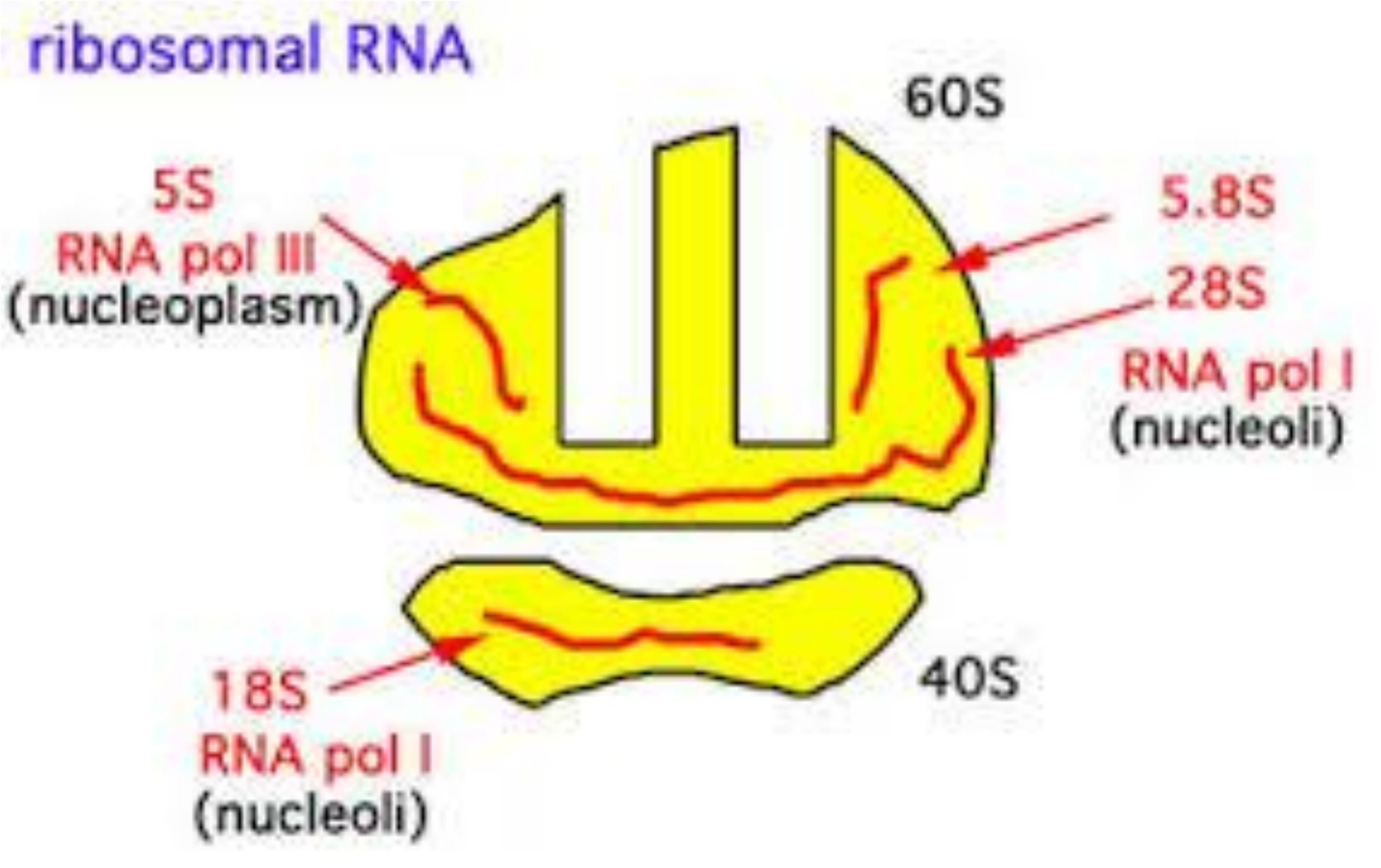
(3) Fundamentals of Biochemistry (Donald Voet, Judith G. Voet and Charlotte W. Pratt) 3rd Edition

trna


3' OH
Amino acid attachment site
Phosphorylated 5' terminus
5' P
TψC loop
DHU loop
UH₂ (Present at one or more indicated sites)
"Extra arm" (variable)
Anticodon loop
Anticodon


Carrier end (amino acid binding site)
C - C - A - CH
Enzyme site
Ribosome site
Anticodon (mRNA recognition end)
G
G
C
C G C C G U C A
mRNA molecule →|Codon|←

Ribosome and Ribosomal RNA



Molecular Interactions

Genetic code:

Def. Genetic code is the nucleotide base sequence on DNA (and subsequently on mRNA by transcription) which will be translated into a sequence of amino acids of the protein to be synthesized.
The code is composed of codons
Codon is composed of 3 bases (e.g. ACG or UAG). Each codon is translated into one amino acid.

The 4 nucleotide bases (A,G,C and U) in mRNA are used to produce the three base codons. There are therefore, 64 codons code for the 20 amino acids, and since each codon code for only one amino acids this means that, there are more than one done for the same amino acid.

How to translate a codon (see table):

This table or dictionary can be used to translate any codon sequence. Each triplet is read from 5′ → 3′ direction so the first base is 5′ base, followed by the middle base then the last base which is 3′ base.

Examples: 5′- A UG- 3′ codes for methionine

5′- UCU- 3′ codes for serine

5′ - CCA- 3′ codes for proline

Termination (stop or nonsense) codons:

Three of the 64 codons; UAA, UAG, UGA do not code for any amino acid. They are termination codes which when one of them appear in mRNA sequence, it indicates finishing of protein synthesis.

Characters of the genetic code:

1- Specificity: the genetic code is specific, that is a specific codon always code for the same amino acid.

2- Universality: the genetic code is almost universal, that is, the same codon is used in all living organisms with few exceptions

3- Degeneracy: the genetic code is degenerate i.e. although each codon corresponds to a single amino acid,one amino acid may have more than one codons. e.g arginine has 6 different codons (give more examples from the table).

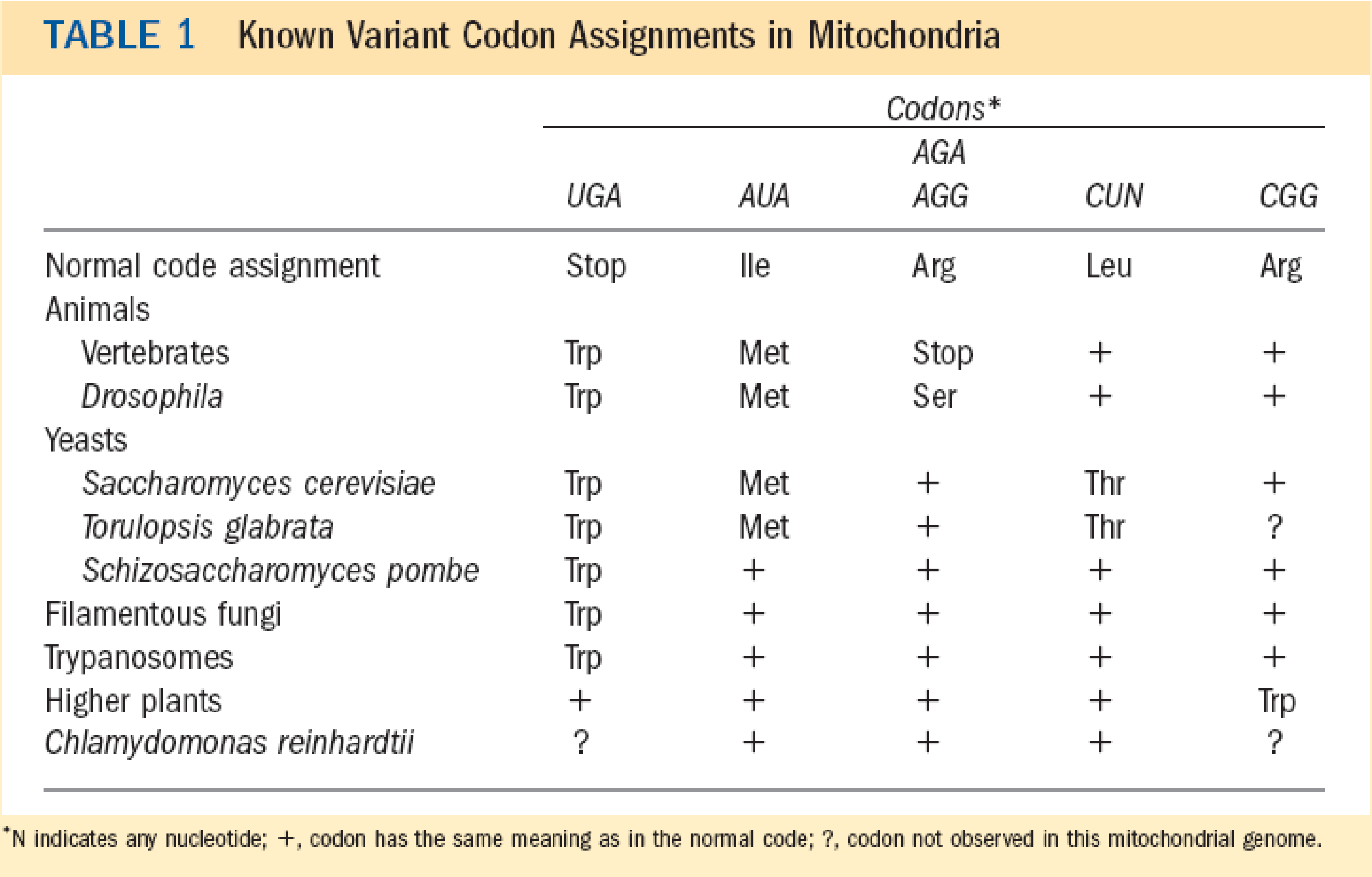
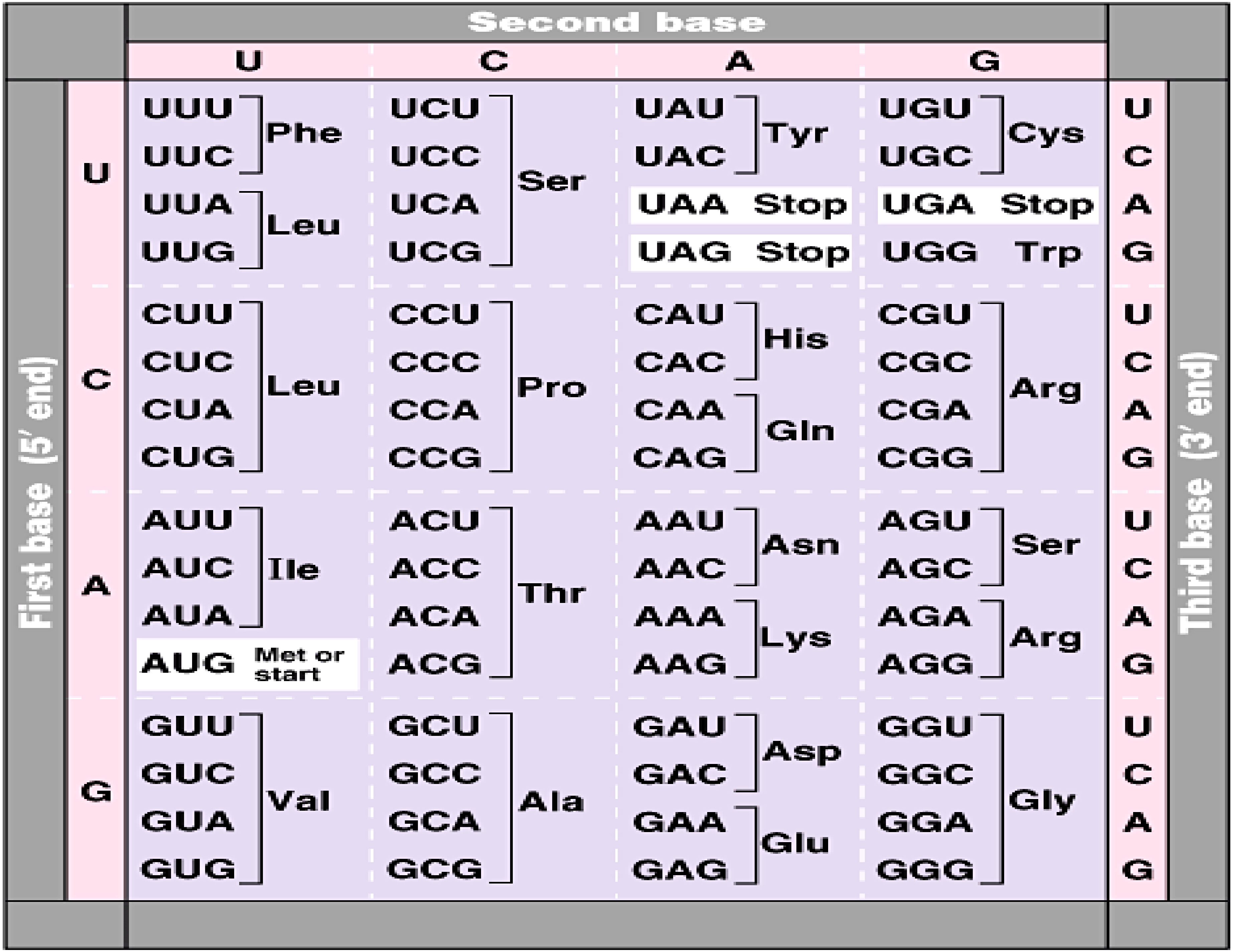


TABLE 1 Known Variant Codon Assignments in Mitochondria

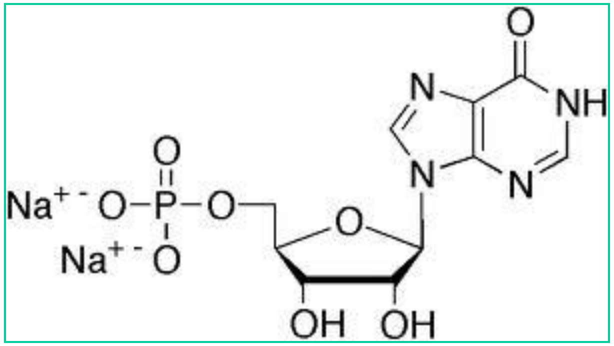
	Codons*				
	UGA	AUA	AGA AGG	CUN	CGG
Normal code assignment	Stop	Ile	Arg	Leu	Arg
Animals					
Vertebrates	Trp	Met	Stop	+	+
Drosophila	Trp	Met	Ser	+	+
Yeasts					
Saccharomyces cerevisiae	Trp	Met	+	Thr	+
Torulopsis glabrata	Trp	Met	+	Thr	?
Schizosaccharomyces pombe	Trp	+	+	+	+
Filamentous fungi	Trp	+	+	+	+
Trypanosomes	Trp	+	+	+	+
Higher plants	+	+	+	+	Trp
Chlamydomonas reinhardtii	?	+	+	+	?

*N indicates any nucleotide; +, codon has the same meaning as in the normal code; ?, codon not observed in this mitochondrial genome.



First base (5′ end)	Second base								Third base (3′ end)
	U		C		A		G		
U	UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys	U
	UUC		UCC		UAC		UGC		C
	UUA	Leu	UCA		UAA	Stop	UGA	Stop	A
	UUG		UCG		UAG	Stop	UGG	Trp	G
C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	Gln	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	Lys	AGA	Arg	A
	AUG	Met or start	ACG		AAG		AGG		G
G	GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	Glu	GGA		A
	GUG		GCG		GAG		GGG		G

Wobble Hypothesis



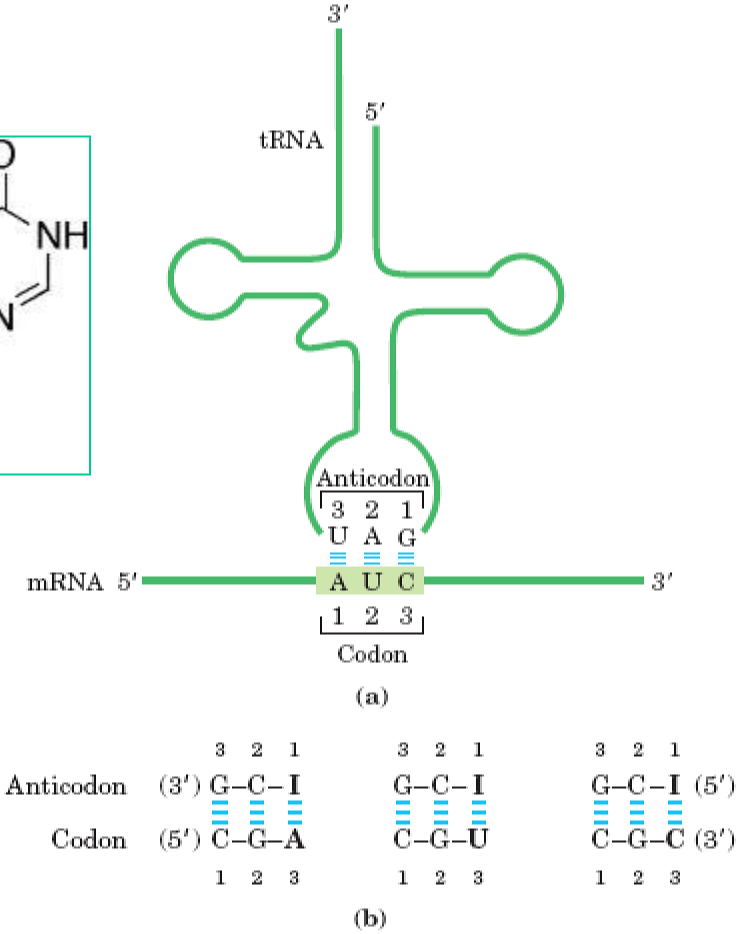


FIGURE 27-8 **Pairing relationship of codon and anticodon.** (a) Alignment of the two RNAs is antiparallel. The tRNA is shown in the traditional cloverleaf configuration. (b) Three different codon pairing relationships are possible when the tRNA anticodon contains inosinate.

Wobble Hypothesis

Crick (1966) proposed' the Wobble hypothesis' in order to solve this appar-ent dilemma. According to this hypothesis, only the first two bases of the codon have a precise pairing with the bases of the anticodon of mRNA, while the pairing between the third bases of codon and anticodon may Wobble (non specific).

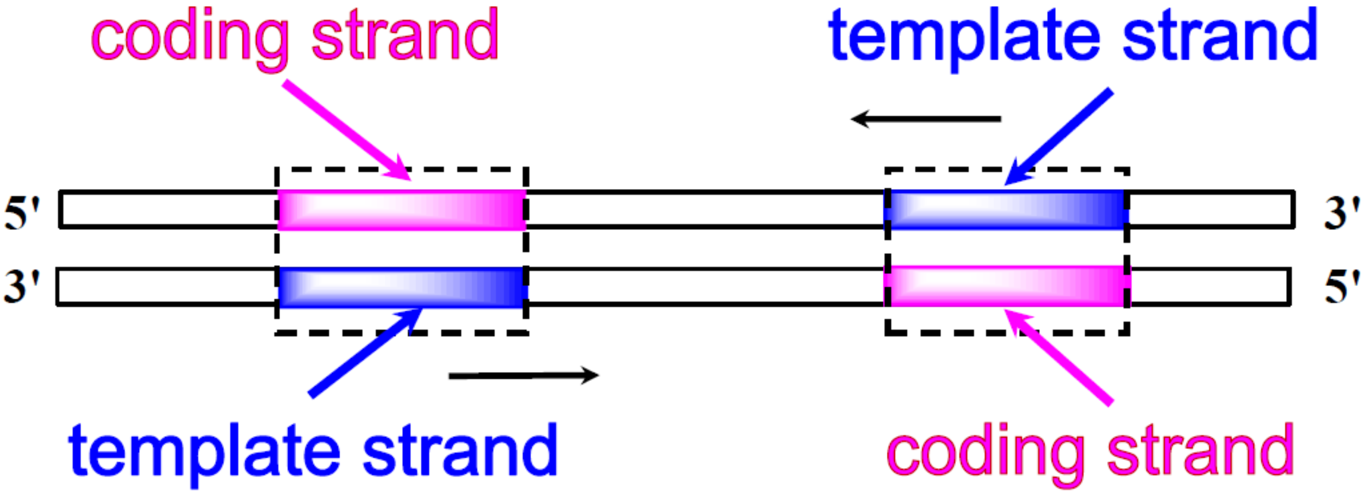
The pairing in the third base is ambiguous. Thus a single tRNA can pair (bind) with more than one mRNA codon differing in only the third base. For example the anticodon ICG of tRNA can read codons AGC and AGU of mRNA.

Wobble Hypothesis

A **wobble base pair** is a pairing between two nucleotides in RNA molecules that does not follow Watson-Crick **base pair** rules.

The four main wobble base pairs are guanine-uracil (**G-U**), hypoxanthine-uracil (**I-U**), hypoxanthine-adenine (**I-A**), and hypoxanthine-cytosine(**I-C**). In order to maintain consistency of nucleic acid nomenclature, "I" is used for hypoxanthine because hypoxanthine is the nucleobase of inosine

What was this??????.....................



The reading frame

A **reading frame** is a sequence of nucleotide triplets that are read as codons specifying amino acids; a single strand of DNA sequence has three possible **reading frames**.

The reading frame

A specific first codon in the sequence establishes the **reading frame.**

In which a new codon begins every three nucleotide residues.

There is no punctuation between codons for successive amino acid residues.

The amino acid sequence of a protein is defined by a linear sequence of contiguous triplets.

In principle, any given single-stranded DNA or mRNA sequence has three possible reading frames.

Each reading frame gives a different sequence of codons but only one is likely to encode a given protein.

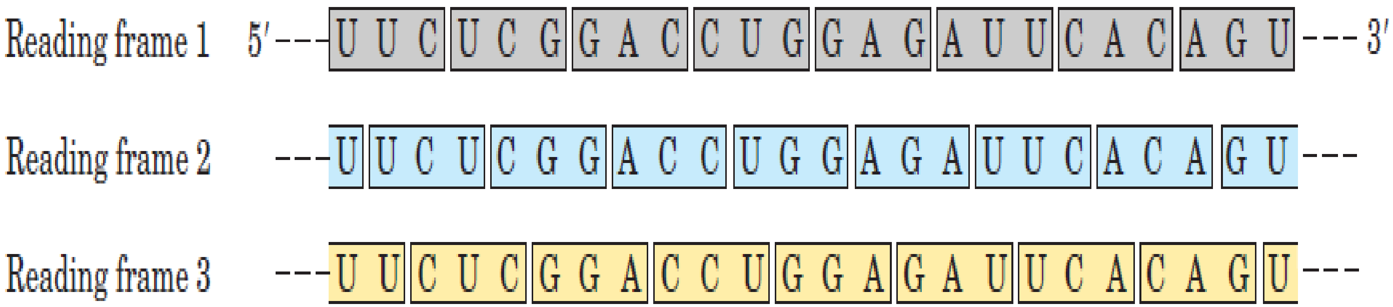

Reading frame 1
5′---UUC UCG GAC CUG GAG AUU CAC AGU---3′
Reading frame 2
---U UCU CGG ACC UGG AGA UUC ACA GU---
Reading frame 3
---UU CUC GGA CCU GGA GAU UCA CAG U---

FIGURE 27-5 Reading frames in the genetic code. In a triplet, nonoverlapping code, all mRNAs have three potential reading frames, shaded here in different colors. The triplets, and hence the amino acids specified, are different in each reading frame.

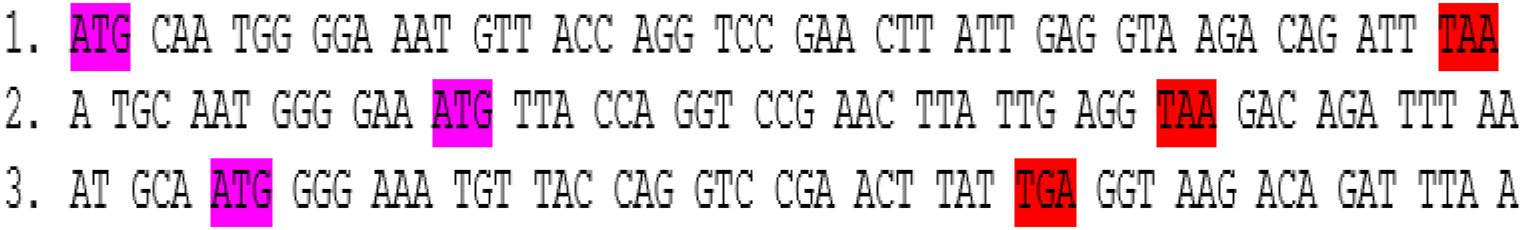
ORFs

- **Open reading frames**. Definition. **Open reading frames** (ORFs) are parts of a **reading frame** that contain no stop codons.
- An **open reading frame** (ORF) is the part of a **reading frame** that has the ability to be translated.
- An ORF is a continuous stretch of codons that contain a start codon (usually AUG) and a stop codon (usually UAA, UAG or UGA)

ORFs

- In general, a reading frame without a termination codon among 50 or more codons is referred to as an **open reading frame (ORF).**
- **Long open reading** frames usually correspond to genes that encode proteins.

ORFs



1. ATG CAA TGG GGA AAT GTT ACC AGG TCC GAA CTT ATT GAG GTA AGA CAG ATT TAA
2. A TGC AAT GGG GAA ATG TTA CCA GGT CCG AAC TTA TTG AGG TAA GAC AGA TTT AA
3. AT GCA ATG GGG AAA TGT TAC CAG GTC CGA ACT TAT TGA GGT AAG ACA GAT TTA A

Mutations

Alterations in DNA structure that produce permanent changes in the genetic information encoded therein.

Gene mutation (altering the nucleotide sequence):

1- Point mutation: changing in a single nucleotide base on the mRNA can lead to any of the following 3 results:

i- Silent mutation: i.e. the codon containg the changed base may code for the same amino acid. For example, in serine codon UCA, if A is changed to U giving the codon UCU, it still code for serine. See table.

ii- Missense mutation: the codon containing the changed base may code for a different amino acid. For example, if the serine codon UCA is changed to be CCA (U is replaced by C), it will code for proline not serine leading to insertion of incorrect amino acid into polypeptide chain.

iii- Non sense mutation: the codon containing the changed base may become a termination codon. For example, serine codon UCA becomes UAA if C is changed to A. UAA is a stop codon leading to termination of translation at that point.

The **termination codons (UAA, UAG, and UGA),** also called stop codons or **nonsense codons**, normally signal the end of polypeptide synthesis and do not code for any known amino acids.

Silent Mutations

ATG	GAA	GCA	CGT
Met	Glu	Ala	Gly

↓

ATG	GAG	GCA	CGT
Met	Glu	Ala	Gly

Missense Mutations

ATG	GAA	GCA	CGT
Met	Glu	Ala	Gly

↓

ATG	GAC	GCA	CGT
Met	Asp	Ala	Gly

Nonsense Mutations

ATG	GAA	GCA	CGT
Met	Glu	Ala	Gly

↓

ATG	TAA	GCA	CGT
Met	STOP		

Types of point mutation:

U A A (termination codon) Nonsense mutation

↑

U C A → U C U Silent mutation

(codon for serine) (codon for serine)

↓

C C A (codon for proline) Missense mutation:

Give other examples on missense mutation which leads to some Hb disease.

2- Frame- shift mutation:

deletion or addition of one or two base to message sequence, leading to change in reading frame (reading sequence) and the resulting amino acid seuence may become completely different from this point.

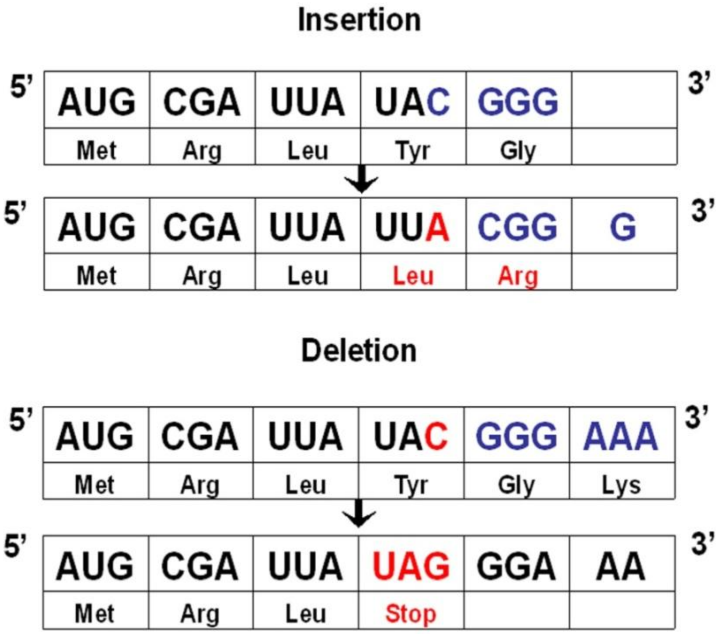

Insertion
5' AUG CGA UUA UAC GGG 3'
Met Arg Leu Tyr Gly
5' AUG CGA UUA UUA CGG G 3'
Met Arg Leu Leu Arg
Deletion
5' AUG CGA UUA UAC GGG AAA 3'
Met Arg Leu Tyr Gly Lys
5' AUG CGA UUA UAG GGA AA 3'
Met Arg Leu Stop

Figure 2. Schematic representation of nucleotide insertion and deletion

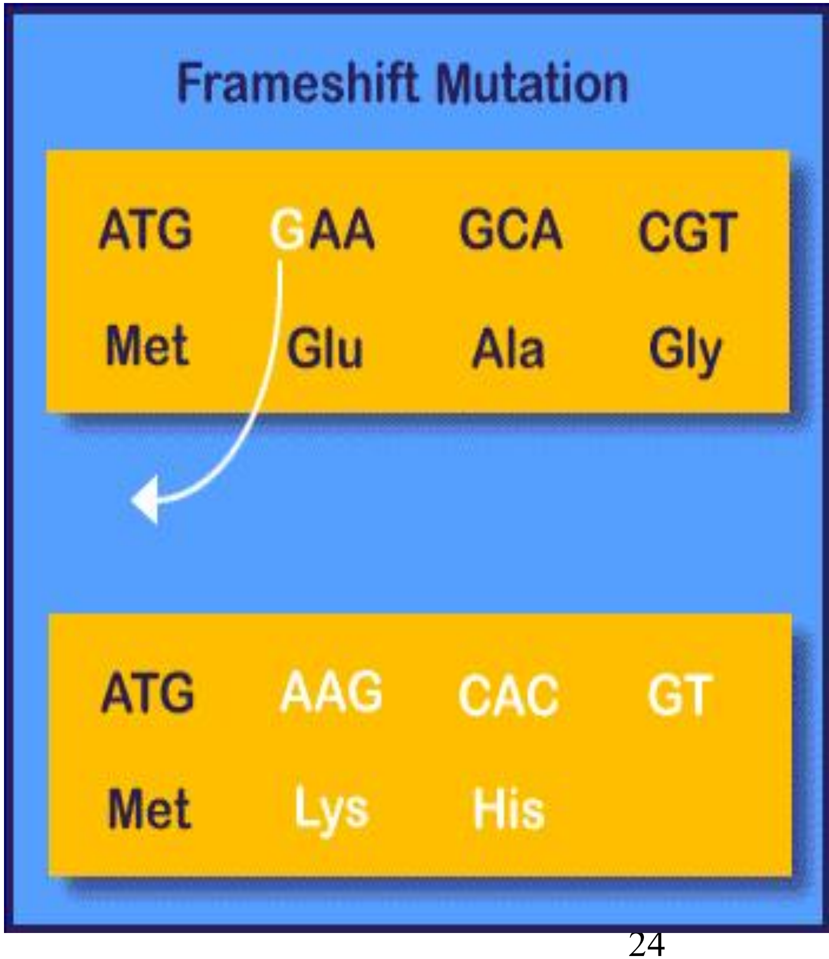
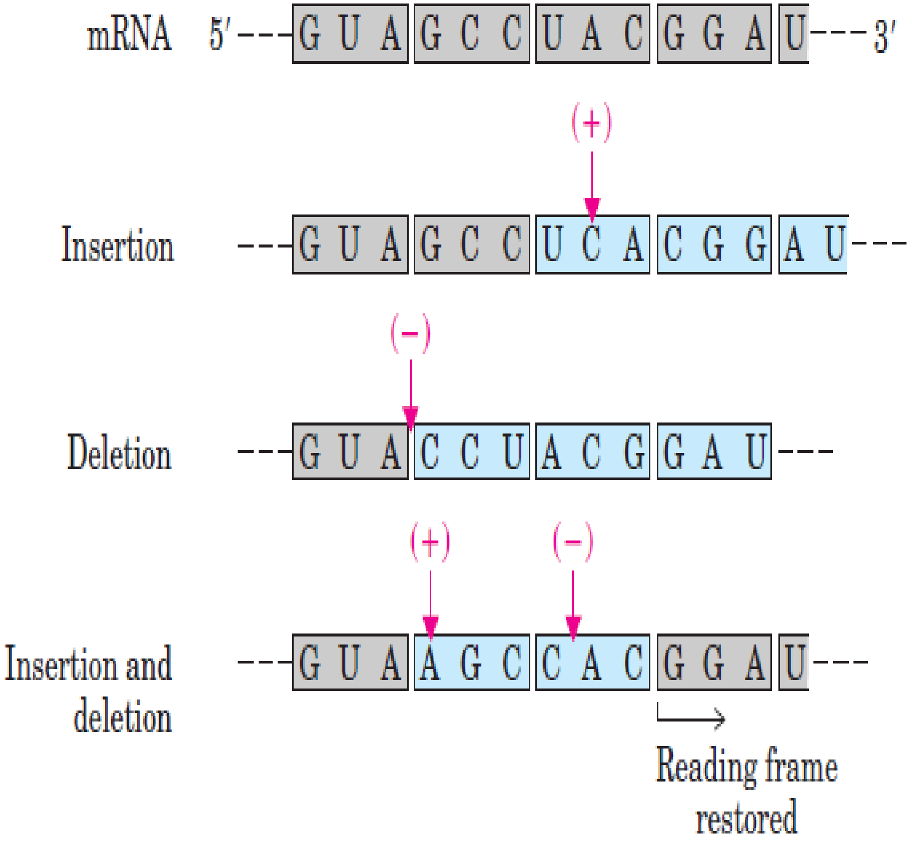


FIGURE 27-4 **The triplet, nonoverlapping code.** Evidence for the general nature of the genetic code came from many types of experiments, including genetic experiments on the effects of deletion and insertion mutations. Inserting or deleting one base pair (shown here in the mRNA transcript) alters the sequence of triplets in a nonoverlapping code; all amino acids coded by the mRNA following the change are affected. Combining insertion and deletion mutations affects some amino acids but can eventually restore the correct amino acid sequence. Adding or subtracting three nucleotides (not shown) leaves the remaining triplets intact, providing evidence that a codon has three, rather than four or five, nucleotides. The triplet codons shaded in gray are those transcribed from the original gene; codons shaded in blue are new codons resulting from the insertion or deletion mutations.


mRNA
5′---G U A | G C C | U A C | G G A | U---3′
(+)
Insertion
---G U A | G C C | U C A | C G G | A U---
(−)
Deletion
---G U A | C C U | A C G | G A U---
(+)
(−)
Insertion and deletion
---G U A | A G C | C A C | G G A | U---
Reading frame restored